vivantis

Flex-C Cloning Kit

MEPL02 , 20 applications
MEPL02-S , 5 applications

User Manual

Description:
Flex-C Cloning Kit is a highly efficient, rapid and easy-to-use PCR cloning kit. The Flex-C Enzyme allows direct cloning of any PCR fragments into any linearized expression vector at any site in a single 20-minute reaction.

The application protocol is simple. The PCR fragments can be generated by PCR Polymerases (for eg. *Taq* DNA Polymerase) with primers that are designed to have at least 10 bases of homology with the vector at their linear ends. No additional treatment of the PCR fragment is required (such as restriction digestion, ligation, phosphorylation, or blunt-end polishing). The linearized vector can be generated by PCR or restriction enzymes (single or double digestion). Flex-C Enzyme joins PCR fragments and linearized vectors accurately and efficiently by recognizing the 10bp overlap at their ends.

This method allows cloning of multiple fragments into a single vector in a single reaction, without subcloning, to create fusion proteins, to delete and replace DNA sequences or to insert point mutations. The Flex-C Cloning Kit is highly efficient with a 95% insert rate. The Flex-C Cloning Kit also includes Vivantis *Taq* DNA Polymerase and reaction buffers as well as dNTPs for subsequent PCR screening of clones.

Key Features:
> Clone any insert, at any site within any vector
> Restriction enzyme, phosphatase and ligase-free system
> Joining multiple fragments at once
> Broad PCR size up to 10kb
> Good for 5' overhangs, 3' overhangs, blunt ends
> Precise insertion at a desired orientation
> High Efficiency with > 95% positive clones
> Multiple applications:
  - adding adaptor, linker and tag before or after the insert
  - mutation generation
  - gene synthesis
> High throughput application

Components:	**MEPL02**	**MEPL02-S**
Flex-C Cloning Enzyme	20 app	5 app
Taq DNA Polymerase	500u	50u
10X ViBuffer A	2ml	1ml
10X ViBuffer S	1ml	1ml
50mM $MgCl_2$	1ml	1ml
10mM dNTPs mix	0.25ml	0.025ml
Nuclease-free Water	1ml	1ml

Storage: Store at -20°C

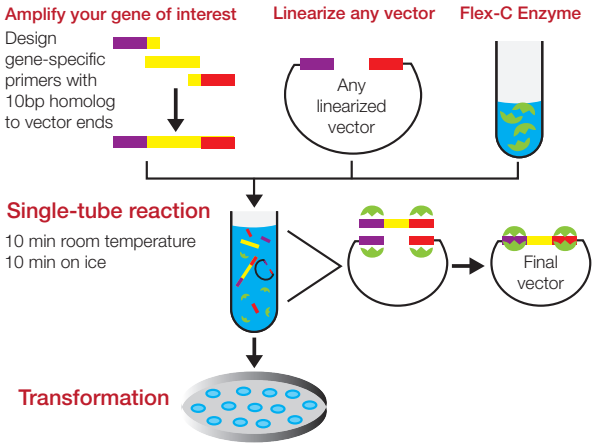
Taq DNA Polymerase Storage Buffer:
20mM Tris-HCl (pH8.0 at 22°C), 100mM KCl, 0.5% Tween™ 20, 0.5% Nonidet-P40, 0.1mM EDTA, 1mM DTT and 50% glycerol.

10X ViBuffer A:
500mM KCl, 100mM Tris-HCl (pH9.2 at 20°C) and 0.1% Triton™ X-100.

10X ViBuffer S:
160mM (NH4)2SO4, 500mM Tris-HCl (pH9.2 at 22°C), 17.5mM $MgCl_2$ and 0.1% Triton™ X-100.

Additional items to be supplied by user:
Gene-specific primers with 10bp homolog to vector ends
Linearised vector
Competent cells
LB plates containing appropriate antibiotic

A) Protocol Overview



B) Preparation of Linearized Vector

A linearized vector can be generated by PCR or restriction enzyme digestion (single or double digestion). A complete digested vector is critical in order to achieve high cloning efficiency. Users are recommended to purify the linearized vector by gel DNA recovery method.

Due to the differences in digestion efficiency, different restriction enzymes will generate different amounts of background. Users are encouraged to perform double digestion to reduce the opportunity of self-ligation of the vector. User may increase the incubation time (4 hours up to overnight, depending on the enzymes properties) for a more complete digestion reaction.

Users can test the background by transforming 10-50ng of purified and linearized vector into competent cells. If the background is high, perform the digestion using more restriction enzymes or prolong the incubation period.

C) Primer Design

Primer design is very crucial for a successful cloning reaction. Users can clone two or more fragments into any linearized vector as long as they share 10 homologous bases at each end. Primers should be designed as per the following:

1. The 5' end of the primer MUST contain 10 bases homologous to 10 bases at one end of the DNA fragment to which it will join (not including the restriction site). That can be the vector or another insert.
2. The 3' end of the primer MUST contain the gene-specific sequence. It can be designed using 18-20 bases with GC content between 40-60%.
3. Melting temperature is calculated based on the 3' gene-specific sequence of the primer instead of the entire primer sequence.
4. Avoid complementary sequences within the primers and between primers.

Please refer to the examples below for primer design.

I) Linearized Vector with 5' Overhang

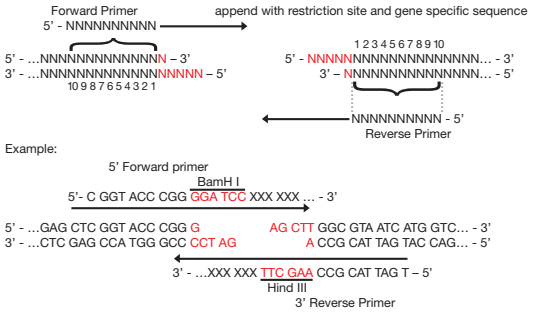


Figure 1: Primer design with restriction site of restriction enzymes generating 5' overhangs.

II) Linearized Vector with 3' Overhang

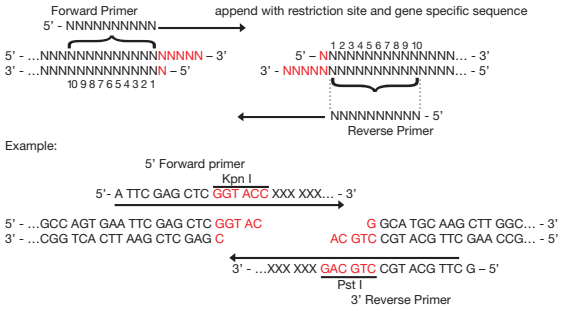


Figure 2: Primer design with restriction site of restriction enzymes generating 3' overhangs.

III) Linearized Vector with Blunt Ends

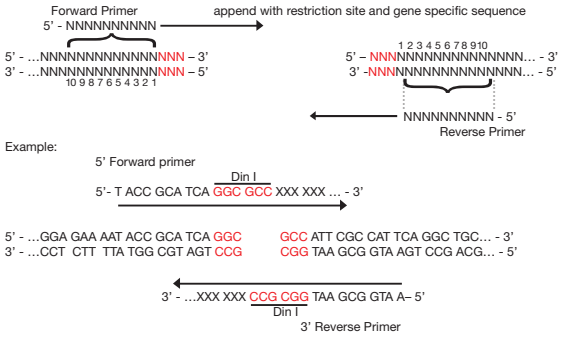


Figure 3: Primer design with restriction site of restriction enzymes generating blunt ends.

Primers above are designed with restriction site of restriction enzymes generating 5' overhang (figure 1), 3' overhang (figure 2) and blunt end (figure 3). Restriction sites are highlighted in red and X represent bases corresponding to the specific gene sequences. The 10 homologous bases are referring to the vector sequences that are adjacent to the restriction sites.

D) PCR Product Consideration

1. We recommend users to use *Taq* DNA Polymerase or other high fidelity polymerase for amplification of gene of interest
2. Purify the PCR product by gel DNA recovery method to remove undesired bands.

E) Cloning Procedure

1. Set up a reaction mixture as follows:

Linearized vector (50-200ng)	2µl
Purified PCR product (20-200ng)	µl*
Flex-C Enzyme Mix	2µl
Nuclease-free water	top up to 10µl
Total Volume	10µl

** Recommended molar ratio for vector and insert is 1:3. In general 1:5 to 3:1 of vector and insert ratio will produce good results.*

2. Incubate at room temperature for 10 minutes and on ice for 10 minutes.
3. Proceed to transformation. Commercial competent cells are recommended.
4. Plate the cells onto LB plates containing appropriate antibiotic for the cloning vector. Incubate all the plates for overnight at 37°C.
5. Perform PCR screening or restriction enzyme digestion to determine the presence of insert.

F) Trouble-shooting

Problem	Possible cause	Solution
Few or no colonies obtainedfrom the transformation	Poor quality competent cells	Test transformation efficiency using supercoiled plasmid DNA (> 10^6 colonies per µg of supercoiled DNA is expected)
	Incorrect primer sequence	Check primer sequences to make it contains 10 homologous bases to the ends of the vector.
	Wrong antibiotics used or with too much antibiotics.	Use media plates with appropriate amount of correct antibiotics.
	Suboptimal PCR product	Use different purification method
	Inhibitory contaminants from PCR product or linearized vector	Both the PCR product and the linearized vector should be purified.
	Transform with too much reaction mixture	Do not add more than 10µl of reaction mixture to 50µl of competent cells. Too much reaction mixture inhibits the transformation.
Colonies do not have insert	Incomplete linearization of vector.	Make sure the vector is completely digested and purify by gel DNA recovery method. Re-digest and purify the vector, if necessary.
	Contamination of other plasmids with same antibiotic resistance	If insert was amplified from a plasmid, circular DNA may have carried through purification and contaminated the cloning reaction. Users are recommended to gel purify the PCR product or the linearizing template DNA.
Colonies contain incorrect insert	Insert contain unspecific sequences.	If the PCR product is not a single distinct band, gel purify the correct insert.